THE PRODUCTION BY STREPTOCOCCUS HEMOLYTICUS OF AN AGGLUTININ FOR RED CORPUSCLES WHICH INHIBITS HEMOLYSIS

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An apparently typical hemolytic streptococcus was isolated from a vegetative growth on an aortic valve at necropsy. Cultures had been made from the ground vegetative growth on human blood-agar plates; and in 36 hours there appeared small, elevated, white, moist colonies, which were surrounded by a clear zone of hemolysis, 2 to 3 mm. in diameter. Films from a colony, stained by Gram's method, showed a small round gram-positive coccus occurring in pairs and in short chains. The coccus was not soluble in bile, nor did it ferment inulin. Classified according to its sugar reactions (Andrews and Horder classification), it belonged to the Streptococcus pyogenes group. The virulence of this streptococcus was tested on white mice, and 0.06 c c of an 18-hour broth culture was found to be the lethal dose.

The ability of this streptococcus to lake blood was further tested by adding 0.5 c c of a 5% sheep corpuscle suspension to each of a series of 10 tubes. Each tube contained 0.5 c c of culture dilutions of an 18-hour broth culture, the amounts being graduated as follows: the first tube, 0.5 c c of culture; the second tube, 0.25 c c, the third tube, 0.125 c c, etc. The tubes were examined after 2 hours' incubation at 37 C., and no hemolysis had occurred. When the tubes were shaken, the corpuscles were found to be agglutinated, in some tubes forming a solid mass which was impossible to break up by shaking the mixture. The tests were repeated under the same and under different conditions. A series of tubes containing uninoculated broth was set up thereafter as a control. At no time were the corpuscles in the plain broth control tubes agglutinated. The following modifications were made in the test: whole defibrinated sheep blood was used instead of the washed corpuscles; human corpuscles, rabbit corpuscles, mouse corpuscles and guinea-pig corpuscles were substituted for the sheep corpuscles; the quantity of 18-hour broth culture was kept a constant, and the amount of blood varied; 24, 48 and 72-hour broth cultures were used instead of the 18-hour broth culture; the incubation period was lengthened. In all of the tests there was marked agglutination.

There was some hemolysis when the tests were incubated for 12 hours, a degree of hemolysis that corresponded with that in the broth control tubes.

An attempt was made to estimate roughly the amount of agglutinin in an 18-hour broth culture by the following method: A tube containing 0.5 c c of the streptococcus broth culture and 0.5 c c of 5% human corpuscle suspension was incubated 1 hour, then centrifugated, and the supernatant removed from the agglutinated corpuscles and incubated with fresh red blood corpuscles for one hour; the fresh corpuscles were agglutinated, and after centrifugation the supernatant fluid agglutinated the third addition of fresh human corpuscles.

In order to determine whether the agglutinin had modified the red blood cells so that they could not readily lake, a fragility test and a hemolytic test were made on the human corpuscles agglutinated by the streptococus broth culture and on fresh human corpuscles. The degree of hemolysis in the tests on the treated corpuscles and in the tests on the fresh corpuscles was practically the same in each instance.

The hemolytic tests were repeated after one month. Corpuscles were partially agglutinated, and partially laked. The streptococcus strain was passed through a series of white mice to discover whether the agglutinin that was decreasing could be increased. The strain had little virulence, 1 c c of the culture failing to kill a mouse. Neither the virulence nor the power to agglutinate increased after mouse passage.

TABLE 1

SUPERNATANT FLUID OF STREPTOCOCCUS CULTURES

Dilutions	Human Corpuscles		Sheep Corpuscles		Guinea-Pig Corpuscles		Rabbit Corpuscles		Mouse Corpuscles	
	H	A	H	A	H	A	H	A	H	A
1:2	+	+	+	+	+	++	+	+++	+	++
1:4	0	++	+	+	0	++	0	+++	+	++
1:8	0	++	+	+	0	++	0	+++	+	++
1:16	0	++	+	+	0	++	0	+++	+	++
1:32	0	++	+	+	0	++	0	+++	+	++
1:64	0	++	+	+	0	++	0	+++	+	++
1:128	0	++	+	+	0	++	0	+++	+	++
1:256	0	++	+	+	0	++	0	++	+	++
1:512	0	++	+	+	0	++	0	++	+	++

\+ = Slight
++ = Partial
+++ = Complete

H = Hemolysis
A = Agglutination

TABLE 2

WASHED STREPTOCOCCUS SUSPENSION

Dilutions	Human Corpuscles		Sheep Corpuscles		Guinea-Pig Corpuscles		Rabbit Corpuscles		Mouse Corpuscles	
	H	A	H	A	H	A	H	A	H	A
0	+++	0	0	++	+	+	+++	0	+++	0
1:2	+++	0	0	++	0	0	+++	0	+++	0
1:4	+++	0	0	++	0	0	+++	0	+++	0
1:8	+++	0	0	++	0	0	+++	0	+++	0
1:16	++	0	0	++	0	0	+++	0	++	++
1:32	++	0	0	++	0	0	+++	0	+	++
1:64	+	0	0	++	0	0	++	++	+	++
1:128	0	+	0	++	0	0	0	++	+	++
1:256	0	+	0	++	0	0	0	++	0	++
1:512	0	+	0	++	0	0	0	++	0	++

\+ = Slight
++ = Partial
+++ = Complete

H = Hemolysis
A = Agglutination

Tested after 4 months, the entire broth culture and the supernatant fluid laked corpuscles in 1 : 4 dilutions. The streptococcus suspension caused no hemolysis. Corpuscles were not agglutinated by the broth cultures, by the filtrate, or by the streptococcus suspension. At this time the strain was not virulent for white mice, rabbits or guinea-pigs

SUMMARY

The streptococcus here considered appeared to be a typical Streptococcus hemolyticus when it was cultivated on blood-agar medium, but it had the peculiar quality of agglutinating instead of laking red blood cells when it was grown in broth medium. It seems possible that, on the blood-agar cultures, agglutination of the corpuscles was prevented by the mechanical factor of the solid medium and that accordingly hemolysis took place as usual. The tests noted suggest that the agglutinin for red blood corpuscles was an exogenous product of the bacterial cell and that hemolysin was probably present, but that its action on corpuscles, for some reason not determined, was inhibited by the agglutinin present. Agglutinin production was a transient quality of this streptococcus strain, since it was present in an appreciable amount for only 6 weeks.